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Patterns of Brain Functional Connectivity within Neurocognitive Subtypes of Autism Spectrum Disorder

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Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

August 2017

Patterns of Brain Functional Connectivity within Neurocognitive Subtypes of Autism Spectrum Disorder

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The University of Texas at Austin, 2017

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Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder. There have been increased efforts to identify meaningful subtypes of ASD based on a variety of measures (e.g., behavioral symptoms, genetic information, etc.). Elucidation of homogenous subtypes may lead to clearer understanding of underlying brain functioning and etiology of ASD. A recent exploratory study aimed to determine whether neuropsychological test data could be used to parse a group of individuals with high-functioning ASD into homogenous "subtypes" based on unique neurocognitive profiles (Wagner, 2014). Results of that study were promising and suggested the emergence of 3 clusters. This subset of individuals with ASD was successfully parsed into smaller more homogenous subgroups based on unique neurocognitive profiles driven by performance on measures of reasoning, receptive language, and learning/memory. Thus, corresponding brain regions were selected for further study in order to explore potential underlying differences in brain functioning across identified clusters. Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) is an emerging neuroimaging tool used to examine functional correlations among spatially distinct brain regions. Previous rs-fcMRI studies examining individuals with ASD have found evidence for altered connectivity; however, results have been inconsistent. Inconsistencies may be related to the heterogeneous nature of ASD and underlying differences in potential

neurocognitive subtypes within ASD samples. The current study aimed to extend preliminary research by comparing patterns of functional connectivity of frontal brain regions, Wernicke's area, and hippocampal regions across previously identified clusters to examine potential differences in underlying brain function. Results indicated The ASD subgroup with above average reasoning and language skills had increased frontal functional connectivity in comparison to other ASD subjects and controls, as well as increased posterior superior temporal gyrus connectivity in comparison to other ASD subjects. The ASD subgroup with below average learning and memory had decreased hippocampal functional connectivity in comparison to controls. However, when ASD subgroups were combined, there were no differences in functional connectivity between ASD and controls. Thus, ASD may be related to unique alterations in functional connectivity networks, however meaningful subgroup differences are easily masked by sample heterogeneity. Identification of neurocognitive profiles may provide diagnostic utility both within the spectrum and between ASD and other disorders. Diagnostic clarification in the form of a "neurocognitive subtype" could provide useful information about cognitive strengths and weaknesses and directions for treatment and intervention planning. Further delineation of the ASD spectrum, including variations in cognitive profiles and related underlying brain networks, may reveal important differences in underlying etiology and response to treatment.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Statement of the Problem

Autism Spectrum Disorder (ASD) is a heterogeneous developmental disorder that represents a broad range of phenotypic symptoms and levels of impairment (American Psychiatric Association, 2013). The Centers for Disease Control and Prevention (CDC) currently estimate that approximately 1 in 68 children are affected with ASD, a number that has risen sharply in the past decade (CDC, 2014). Increased awareness, changes in diagnostic criteria, and improvements in identification may be contributing factors to the increased prevalence of the disorder (Fombonne, 2003; Fombonne, Quirke, & Hagen, 2009; Wing & Potter, 2002). Despite decades of research, no clear cause of ASD has been identified (Abrahams & Geschwind, 2010; Geschwind, 2011). Previous research has shown strong support for genetic involvement (Egger et al., 2014) likely interacting with environmental factors (Gardener, Spiegelman, & Buka, 2009; Hunter, 2005). Additionally, twin studies have demonstrated a heritability rate of over 80% (Ronald & Hoekstra, 2011). Recent advances in technology have aided researchers in beginning to isolate genetic variations associated with the disorder (Geschwind, 2011; Murdoch & State, 2013); however, currently no single genetic marker accounts for more than 1-2% of ASD variability (Ellegood et al., 2015).

Heterogeneity of the ASD population exists across the diagnostic symptoms: social skills, communication, restricted interests, and repetitive behavior (Hu & Steinberg, 2009); across cognitive abilities (Lewis, Murdoch, & Woodyatt, 2007; Rapin,

Dunn, Allen, Stevens, & Fein, 2009); and across brain imaging and histology findings (M. Hrdlicka et al., 2005; Rane et al., 2015). Despite the well-established heterogeneity of ASD, the authors of the 5th Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) have eliminated previously defined subtypes (i.e., autistic disorder, Asperger's disorder, PDD-NOS). Clinicians now classify all variations using one diagnostic label: Autism Spectrum Disorder (American Psychiatric Association, 2013). In justification for these revisions, authors of the DSM-5 cite the lack of research supporting a clear distinction between the previous DSM-IV TR diagnostic categories (C. Lord et al., 2012). For example, previous studies have described the ambiguity between Asperger's disorder and high-functioning autistic disorder, with the same symptom presentation receiving a different diagnosis from one clinician to the next (C. Lord et al., 2012). Additionally, numerous brain imaging studies have failed to demonstrate significant differences between Asperger's disorder and high-functioning autistic disorder (Pina-Camacho et al., 2012). In fact, it had become common methodological practice for researchers to combine the Asperger's disorder and high functioning autism subtypes into one group with which to compare to a typically developing control group. Given the difficulty of differentiating between subtypes in both research and clinical practice, the authors of the DSM-5 took a step back, so-to-speak, and thus paved the way for researchers to develop new ways to classify ASD into more stable and meaningful subtypes (R. Grzadzinski, M. Huerta, & C. Lord, 2013).

Perhaps the most significant barrier to identifying the etiology of ASD is the heterogeneity of the disorder (Amaral, 2011). Research findings within ASD are often

inconclusive and inconsistent across studies. For example, a longitudinal analysis of children with ASD found that 40% of children had an atypically rapid growth of the amygdala; however the remaining 60% of children demonstrated amygdala growth at rates similar to or slower than typically developing controls (Nordahl et al., 2010). While the variability of findings within ASD research may be related to methodological differences, study limitations, sample size, regional specificity, or developmental factors, the impact of population heterogeneity has become increasingly discussed. Many researchers within the field are advocating for continued efforts to delineate the population into more homogenous subgroups (Ecker, Spooren, & Murphy, 2013; Rebecca Grzadzinski, Marisela Huerta, & Catherine Lord, 2013; Happe, Ronald, & Plomin, 2006; Happé, Ronald, & Plomin, 2006).

Despite the well-established phenotypic heterogeneity of ASD, research designs have often examined the population as one homogenous group with methodological approaches focused on mean group comparisons to a typically developing control group. Importantly, however, this may mask underlying variability within the spectrum of ASD, resulting in inconsistent research findings and difficulty in making progress towards identification of etiology and effective treatment (Ecker et al., 2013). For example, alterations in brain functioning may exist in a subset of individuals with ASD, but the same alterations may be absent in others or instead may be present in different brain regions. When data are averaged across the spectrum of ASD, these "sub-group" differences may be lost and thus important information about underlying brain functioning may be obscured, resulting in inaccurate and inconsistent findings. Thus, it is

imperative for researchers to continue to strive towards elucidation of more homogenous subtypes within ASD in order to better understand the disorder and facilitate more nuanced examinations that may otherwise be masked by averaging group differences (Lai, Lombardo, Chakrabarti, & Baron-Cohen, 2013).

One possible means for achieving subgroup delineation is through examination of variations in cognitive functioning among individuals with ASD. Clarification of cognitive dysfunction within the population of ASD is important for treatment efforts. Numerous studies have been dedicated to better understanding the range of behavioral, social, and communication functioning across the spectrum of ASD, yet there is a paucity of research examining variations of cognitive abilities within this population. Not surprisingly, the studies that have examined cognitive abilities within ASD have provided inconsistent results. Individuals with ASD have a diverse range of cognitive abilities and disabilities and thus, attempting to define one single cognitive profile of ASD may not be realistic. To date, no single model that has been put forth has fully captured the range of cognitive heterogeneity in the population (Charman et al., 2010). This difficulty may be partially attributed to the fact that these unitary models of cognitive profiles have often been described by mean deficits across a heterogeneous sample, thus obscuring different patterns of spared/impaired cognitive functioning that may exist in subgroups of individuals with ASD.

PRELIMINARY CLUSTERING STUDY

A recent exploratory study aimed to determine whether neuropsychological test data could be used to parse a small group of individuals with high functioning ASD into

smaller and more homogenous subgroups based on neurocognitive profiles (Wagner, 2014). This preliminary study examined 20 young adult males between the ages of 18 and 24 years old. Data included neuropsychological test scores across 12 domains of cognitive functioning: general cognitive ability, visuospatial processing, verbal learning and memory, visual learning and memory, working memory, reasoning, cognitive flexibility, attention, receptive language, expressive language, social and emotional processing, and fine motor skills. To determine whether unique profiles of neuropsychological functioning exist among individuals with ASD, as well as the nature of these potential subgroups, an initial hierarchical clustering analysis was followed by a *k*–means cluster analysis. Results of that study were promising and suggested the emergence of 3 clusters with unique strengths and weaknesses. Of the total sample of 20 participants, nine belonged to cluster 1 (C1); nine belonged to cluster 2 (C2); and two belonged to cluster 3 (C3). Mean domain scores for each cluster are illustrated in **Figure C1**. C1 included subjects with strengths in general intellectual ability (FSIQ) and reasoning, high average receptive and expressive language, and a weakness in fine-motor skills. C2 was defined by subjects with high FSIQ scores and low scores on measures of verbal learning and memory, visual learning and memory, and fine-motor skills. Generally, performance across domains appeared more varied in this cluster. C3 was comprised of 2 subjects with relatively low performance on tests of reasoning ability, cognitive flexibility, working memory, verbal learning and memory, expressive and receptive language, and visuospatial processing.

The relative importance of each composite domain score in the final cluster solution was determined using analysis of variance (ANOVA) techniques. Examination of the resulting ANOVA (see **Table C1**) suggests the reasoning domain score provided the greatest separation between clusters and thus contributed most heavily to the final cluster solution, followed by performance on tests of receptive language and verbal learning/memory. Interestingly, performance on measures of social and emotional processing was less useful for determining the final cluster solution and subjects tended to perform in the average range in this domain across the clusters.

The findings suggest a methodology for parsing a heterogenous group of individuals with ASD into smaller and more meaningful subgroups. Variation in cognitive ability among the 3 clusters may be related to variations in underlying brain structure or functioning. The neuropsychological domains that contributed most heavily to the final cluster solution were reasoning, receptive language, and verbal learning/memory. Thus, exploration of corresponding brain regions (i.e., frontal brain regions, superior temporal gyrus, and hippocampal regions) may reveal underlying differences in brain functioning among identified subgroups.

Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) is an emerging neuroimaging tool used to examine the functional coherence between spatially distinct brain regions. A common approach involves identification of "seed regions" to explore coherence in low-frequency blood oxygen level dependent (BOLD) signal between the identified seed region/s and other areas of the brain. Previous rs-fcMRI studies have found evidence for altered functional connectivity in ASD; however,

results have been inconsistent (Muller, 2007; Rane et al., 2015). Inconsistencies between studies may be related to the heterogenous nature of ASD and underlying differences in potential neurocognitive subtypes within the ASD samples.

Purpose of the Current Study

The current study aimed to extend this author's preliminary research by comparing the brain imaging data of individuals in previously identified clusters in order to examine potential differences in underlying functional connectivity. Specifically, the current study examined functional connectivity patterns of brain regions related to the domains that contributed most heavily to the final clustering solution (i.e., reasoning = middle frontal gyrus, receptive language = posterior superior temporal gyrus, and verbal learning/memory = hippocampus) among previously identified neurocognitive subtypes of ASD. In addition to providing validation of previously identified clusters, the current study aimed to further recent attempts to unravel the heterogeneity within this population by providing additional information regarding differences in underlying functional connectivity in individuals with ASD.

Research Questions and Hypotheses

Research Question 1: Do C1 and C2 subgroups display altered patterns of functional connectivity relative to each other and control subjects in networks associated with reasoning, receptive language, and learning/memory?

Hypothesis 1A: C1 will display altered patterns of functional connectivity in networks associated with reasoning relative to both C2 and controls, who will not differ from each other.

Rationale: C1 included subjects with high performance on measures of reasoning. C2 included subjects with average performance on measures of reasoning.

Hypothesis 1B: C1 will display altered patterns of functional connectivity in networks associated with receptive language relative to both C2 and controls, who will not differ from each other.

Rationale: C1 included subjects with high performance on measures of receptive language. C2 included subjects with average performance on measures of receptive language.

Hypothesis 1C: C2 will display altered patterns of functional connectivity in networks associated with learning and memory relative to both C1 and controls, who will not differ from each other.

Rationale: C2 included subjects with low performance on measures of learning and memory. C1 included subjects with average performance on measures of learning and memory.

Research Question 2: Do patterns of functional connectivity in a combined ASD group (C1 and C2) significantly differ from controls subjects in networks associated with reasoning, receptive language, and learning/memory?

Hypothesis 2: The ASD combined group will not significantly differ from the control group.

Rationale: When C1 and C2 are combined into one ASD group, the differences in C1 relative to controls and in C2 relative to controls will be averaged out and thus significant alterations in functional connectivity will be masked.

Chapter 2: Methods

PARTICIPANTS AND PROCEDURES

Neurocognitive data and brain imaging data were previously collected as part of a larger study examining the anatomical and functional connectivity of the cerebellum in autism spectrum disorder (ASD). All data were de-identified prior to the current study. Data collections procedures for the larger study are briefly reviewed here.

Subjects were recruited into the larger study via professional recommendation and self-referral as well as advertising through various agencies, conferences, schools, and websites. All subjects gave informed consent prior to testing, and were compensated for their time. The University of Texas at Austin Institutional Review Board approved all procedures. Inclusion criteria required that subjects be between 18 and 26 years old and speak English as their primary language. Participants were excluded if they had an IQ (as measured by the Wechsler Abbreviated Scales of Intelligence [WASI]) of <70, a known history of epilepsy, mental retardation, fragile X syndrome, other major psychiatric or neurologic diagnosis, experienced a significant head injury that involved loss of consciousness for greater than 30 minutes, or had any significant physical or psychiatric disability that prevented involvement in the study.

Subjects in the ASD group were evaluated by a psychologist with expertise in autism diagnosis prior to further testing. Confirmation of ASD diagnosis, using DSM-IV diagnostic criteria (American Psychiatric Association, 2013), was determined using the Autism Diagnostic Interview-Revised (ADI-R) (Catherine Lord, Rutter, & Couteur,

1994) and the Autism Diagnostic Observation Schedule (ADOS) (Catherine Lord et al., 1989).

Participants in the current study included 18 young adult males between the ages of 18 and 24 years old (M = 21.140, SD = 2.197) who participated in the preliminary exploratory study (Wagner, 2014) and 18 matched controls. Specifically, the 9 subjects from cluster 1 (C1) and 9 subjects from cluster 2 (C2) were included in the analyses. The 2 subjects from cluster 3 (C3) were not included due to the limited sample size. Additional information regarding the methodology and results of the preliminary clustering study (detailed in Wagner, 2014) will be summarized below.

NEUROCOGNITIVE MEASURES

Participants were evaluated using standardized assessments and administration procedures across 12 domains of neurocognitive functioning: general cognitive ability, visuospatial processing, verbal learning and memory, visual learning and memory, working memory, reasoning, cognitive flexibility, attention, receptive language, expressive language, social and emotional processing, and fine motor skills. For information on tests included within each domain, see **Table C2**. All tests are considered to have high reliability and validity (Strauss, Sherman, & Spreen, 2006).

IMAGING DATA ACQUISITION

Magnetic resonance imaging (MRI) data were collected on a General Electric Signa Excite 3.0 Tesla HD scanner. Structural and functional data for each participant were collected during the same scan session. Resting state images were acquired with a gradient echo planar imaging (EPI) pulse sequence (TR = 2000 ms; TE = 30 ms; flip

angle = 90°; 46 3.0-mm axial slices; matrix = 64 x 64; FOV = 240 mm; in-plane resolution = 3.75 mm x 3.75 mm; duration 7 min 14 sec) while subjects were instructed to rest. A time series of 216 EPI volumes were acquired for each participant. Structural data consisted of high-resolution sagittal images (SPGR sequence: TR = 6.1 ms; TE = 1.3 ms; flip angle = 11°; matrix = 256 x 256; FOV = 256 mm; slice thickness = 1 mm).

PRELIMINARY CLUSTERING ANALYSES

Full details regarding the preliminary clustering analyses and results can be found in Appendix B, and are briefly summarized here. Data from all neuropsychological measures were transformed into composite z-scores reflecting each domain of interest, resulting in 12 composite mean z-scores. An initial agglomerative hierarchical clustering algorithm was followed by a *k*–means cluster analysis. The nature of the clusters identified during the *k*–means cluster analysis was defined by examining the means of the final cluster centers for each of the 12 domains. The relative importance of each composite domain score in the final cluster solution was determined using analysis of variance (ANOVA) techniques.

Preliminary Clustering Results

Based on examination of the dendrogram resulting from the initial hierarchical cluster analysis, the three-cluster solution was selected as providing the best separation between clusters. Next, a *k*–means cluster analysis was run and final cluster centers were achieved with convergence after three iterations. Mean domain scores for each cluster are illustrated in **Figure C1**. Of the total sample of 20 participants, nine belonged to cluster 1 (C1); nine belonged to cluster 2 (C2); and two belonged to cluster 3 (C3).

C1 included subjects with strengths in general intellectual ability (FSIQ) and reasoning, high average receptive and expressive language, and a weakness in fine-motor skills. C2 was defined by subjects with high FSIQ scores and low scores on measures of verbal learning and memory, visual learning and memory, and fine-motor skills. Generally, performance across domains appeared more varied in this cluster. C3 was comprised of 2 subjects with relatively low performance on tests of reasoning ability, cognitive flexibility, working memory, verbal learning and memory, expressive and receptive language, and visuospatial processing. Examination of the resulting ANOVA table (see **Table C1**) suggests the reasoning domain scores provided the greatest separation between clusters and thus contributed most heavily to the final cluster solution, followed by performance on tests of receptive language and verbal learning and memory.

IMAGING ANALYSES

Imaging data pre-processing

Data preprocessing was completed using the CONN toolbox, a Matlab/SPM-based cross-platform software for the computation, display, and analysis of functional connectivity MRI data. First, structural and functional images were visually inspected for movement or other artifact and flagged for potential censoring during later analyses. Preprocessing of functional and anatomical volumes was carried out using CONN's default MNI pipeline, which consisted of the following steps: functional realignment and unwarping, slice-timing correction, coregistration, structural segmentation and normalization, functional normalization, outlier detection, and smoothing.

First, initial volumes that were collected prior to signal stabilization were removed. This included the first 2-4 volumes. Then, mean absolute deviations at each time point were calculated to identify volumes with a high percentage of outlier voxels. Next, slice-time correction was applied to minimize error related to time sampling. Due to images being collected one slice at a time, each slice has a slightly different time of sampling. Slice-time correction accounts for this by interpolating all of the slices as though they were acquired at the beginning of each time point (Huettel, Song, & McCarthy, 2004). Then, motion correction was carried out using volume registration to correct for participant movement in the scanner, which otherwise may alter the time course of each voxel measurement (Jenkinson, Bannister, Brady, & Smith, 2002). Movement along X-, Y-, and Z-axis was calculated along with rotation in roll, pitch, and yaw. Volume registration corrects movement by adjusting to a reference volume. Importantly, while motion correction helps to mitigate spikes in signal related to movement, all motion related activity is not totally accounted for. Therefore, to address variance related to movement, all six motion parameters were included in the regression analysis. The next step in pre-processing involved spatial smoothing to statistically increase the normality of the data (Huettel, et al., 2004). Spatial smoothing calculates a new value for the center of each voxel using the average of surrounding voxels thereby increasing the signal to noise ratio (Gaussian kernel of FWHM 8mm). Functional connectivity research has demonstrated that functional coherence between spatially distinct regions exists within low-frequency time series (Biswal, et al., 1995). Thus, it is commonly recommended to remove frequencies greater than 0.08 Hz. Gaussian low-pass

temporal filtering was applied to remove high-frequency noise (>0.08 Hz) unrelated to signals of interest (Uddin et al., 2009). Next, data were scaled, relative to the mean of all blurred data sets, in order to move arbitrary MRI signal units onto the same scale for each subject. Lastly, in order to compare patterns of connectivity among participants, data were spatially normalized using automated alignment and adjustment to the Montreal Neurological Institute and Hospital (MNI) coordinate system. Data were visually inspected to verify accurate spatial normalization and were flagged for later adjustment, as needed.

Functional Connectivity Analyses

Resting state signals of interest were further isolated by removing nuisance covariates, global signal, and signals from white matter and cerebrospinal fluid. Previous research has shown that the global signal is related to physiological processes such as respiratory and cardiac patterns, thus inclusion as a covariate allows for removal of these fluctuations from the results (Birn, Diamond, Smith, & Bandettini, 2006; O'Reilly et al., 2010; Uddin et al 2009). White matter and cerebrospinal fluid was also regressed out to minimize the inclusion of non-gray matter voxels and non-tissue voxels that might otherwise confound the results of the functional analysis between regions of interest (ROI) and other areas of the brain (Smith et al., 2004; O'Reilly et al., 2010).

For each participant, ROIs included regions thought to correspond to each domain driving the cluster analysis. Because the extent to which individuals with ASD may demonstrate altered patterns of functionally connectivity is not well understood, bilateral ROIs were extracted for each domain. Specifically, ROIs included: left and right middle

frontal gyrus, left and right posterior superior temporal gyrus, and left and right hippocampus. Each region of interest (ROI) was defined using CONN's built-in atlas of cortical and subcortical areas from the FSL Harvard-Oxford Atlas. Temporally filtered time series data were then extracted from all voxels within each seed ROI and averaged to create the mean time series for each ROI (e.g., mean time series of left posterior superior temporal gyrus). Next, to identify areas of functional connectivity between the regions of interest and all other areas of the brain, whole-brain voxel-wise correlations associated with the mean time series for each seed ROI were derived. Fisher's *z*-transformation were used to convert correlation maps to *z*-score maps for each ROI and each subject. Within-group 2-tailed *t* tests were used to identify brain regions where C1, C2, and control group data were significantly different from zero. In order to correct for multiple comparisons, a voxel-level height-threshold of $p < .001$ was used as a cut-off for minimum significance of individual voxels to be retained. Second, at the cluster-level, an extent-threshold of FDR-corrected $p < .05$ was used for limiting which clusters were considered in the results. General convention within the field that suggests a combination of threshold values that combines both height-threshold and extent-thresholds among the values is considered appropriate for false positive control for multiple comparisons. However, the sensitivity/specificity of the results may vary. Use of more liberal height threshold in conjunction with more conservative extant threshold allows for adequate sensitivity when the expected effects may be broad but weak (Friston et al., 1994).

Group Comparisons

To answer the first research question regarding the extent to which connectivity between ROIs and all other areas of the brain differs between groups, group level comparisons were conducted separately for each ROI using two-sample t-tests. This allowed for identification of potential significant positive or negative contrasts between (i) C1 and Controls, (ii) C2 and Controls, and (iii) C1 and C2 in networks of connectivity between seed regions associated with reasoning, learning/memory, and receptive language and all other areas of the brain. Then, C1 and C2 were joined to create a combined ASD group. In order to answer the second research question regarding comparisons between Control subjects and the combined ASD group, additional follow-up t-tests were conducted for each ROI examining potential significant positive or negative contrast between (iv) ASD and Controls in connectivity between seed regions and all other areas of the brain. All group level comparisons were corrected using a height-threshold of $p < 0.001$, cluster-defining threshold of $\leq .05$ alpha (cluster-size p-FDR correction).

Chapter 3: Results

RESEARCH QUESTION 1

Middle Frontal Gyrus Connectivity

Seed regions of interest associated with reasoning included the left and right middle frontal gyrus. Results indicated that, within the Control group, the mean time series of the left middle frontal gyrus was significantly correlated with proximal regions including the left inferior frontal gyrus (pars opercularis and pars triangularis), superior frontal gyrus, and frontal pole (peak voxel -46, 22, 26; p-FDR < 0.000), as well as the left paracingulate gyrus (peak voxel 00, 30, 46; p-FDR < 0.000) and more distal regions in the left posterior supramarginal gyrus (peak voxel -42, -46, 36; p-FDR < 0.000) and left angular gyrus (peak voxel -36, -60, 30; p-FDR = 0.001). Significant contralateral correlations were obtained in areas of the right middle frontal gyrus extending into the frontal pole (peak voxel 44, 32, 30; p-FDR < 0.000), as well as the right cerebellum 6 extending slightly into the cerebellum crus 1 (peak voxel 12, -64, -30; p-FDR = 0.007).

The mean time series of the right middle frontal gyrus within the Control group showed significant correlations with proximal regions including the right frontal pole, inferior frontal gyrus (pars opercularis and pars triangularis), precentral gyrus, frontal operculum, and superior frontal gyrus (peak voxel 44, 26, 22; p-FDR < 0.000), as well as more distal areas of the right posterior supramarginal gyrus and angular gyrus (peak voxel 44, -46, 32; p-FDR < 0.000), and right superior lateral occipital cortex (peak voxel 34, -60, 26; p-FDR = 0.007). Significant contralateral correlations were obtained in areas of the left middle frontal gyrus (peak voxel -42, 22, 32; p-FDR < 0.000) and posterior supramarginal gyrus (peak voxel -50, -50, 26; p-FDR < 0.000), as well as the left

cerebellum 8 and 7b extending into the cerebellum crus 2 (peak voxel -24, -76, -50; p-FDR < 0.000).

Group Comparisons

Consistent with hypothesis 1A, in regions associated with reasoning, C1 demonstrated increased functional connectivity when compared to Controls between the left middle frontal gyrus and a small area within the temporal occipital fusiform cortex extending slightly into the temporooccipital portion of the left inferior temporal gyrus (peak voxel -40, -58, -18; p-FDR = 0.016; **Fig. 2**). C1 also demonstrated increased functional connectivity when compared to C2 between the left middle frontal gyrus and a small area within the right middle frontal gyrus (peak voxel 44, 22, 46; p-FDR = 0.043; **Fig. 3**). Again consistent with hypothesis 1A, there were no significant group differences between C2 and Control for the left middle frontal gyrus seed region. There were no significant between group effects for functional connectivity of the right middle frontal gyrus seed region.

Posterior Superior Temporal Gyrus Connectivity

Seed regions of interest associated with receptive language included the left and right posterior superior temporal gyrus. Results indicated that, within the Control group, the mean time series of the left posterior superior temporal gyrus was significantly correlated with proximal regions extending to the left posterior middle temporal gyrus, posterior supramarginal gyrus, planum temporale, planum polare, central opercular cortex, middle temporal gyrus, and Heschl's gyrus (peak voxel -58, -28, 00; p-FDR < 0.000). Significant contralateral correlations were obtained in areas of the right posterior

superior temporal gyrus, middle temporal gyrus, planum temporale, parietal operculum cortex, and Heschl's gyrus (peak voxel 44, -34, 02; p-FDR < 0.000), as well as a small area within the right inferior lateral occipital cortex (peak voxel 34, -80, -04; p-FDR = 0.025).

The mean time series of the right posterior superior temporal gyrus within the Control group showed significant correlations with proximal regions extending into the right posterior middle temporal gyrus, anterior superior temporal gyrus, posterior supramarginal gyrus, planum temporale, and planum polare (peak voxel 48, -24, -04; p-FDR < 0.000), as well as more distal areas of the right inferior frontal gyrus (pars triangularis) extending into the frontal operculum gyrus (peak voxel 44, 26, 06; p-FDR = 0.004). Significant contralateral correlations were obtained in areas of the left posterior superior temporal gyrus, anterior superior temporal gyrus, posterior middle temporal gyrus, and planum polare (peak voxel -46, -20, -10; p-FDR < 0.000), as well as a small area within the left frontal pole (peak voxel 00, 66, 22; p-FDR = 0.007).

Group Comparisons

Consistent with hypothesis 1B, in regions associated with receptive language, C1 demonstrated increased functional connectivity when compared to C2 between the right posterior superior temporal gyrus and two regions: the pars opercularis of the left inferior frontal gyrus extending slightly into the left fronto-central operculum cortex (peak voxel -50, 10, 00; p-FDR = 0.029; **Fig. 4**), as well as an area in the left posterior temporal lobe (peak voxel -42, -46, 02; p-FDR = 0.029; **Fig. 4**). Additionally, consistent with hypothesis 1B, there were no significant between group differences between C2 and Controls. However, contrary to hypothesis 1B, there were also no significant between

group differences between C1 and Controls. There were no significant group effects for functional connectivity of the left posterior superior temporal gyrus seed region between C1, C2, or Controls.

Hippocampal Connectivity

Seed regions of interest associated with learning and memory included the left and right hypocampus. Results indicated that, within the Control group, the mean time series of left hippocampus was significantly correlated with proximal regions extending into the left amygdala, anterior parahippocampal gyrus, posterior cingulate gyrus, temporal pole, and lingual gyrus (peak voxel -20, -42, -04; p-FDR < 0.000). Significant contralateral correlations were obtained in areas of the posterior parahippocampal gyrus and temporal occipital fusiform cortex (peak voxel 38, -38, -10; p-FDR < 0.000), as well as the right hippocampus and anterior parahippocampal gyrus (peak voxel 26, -20, -20; p-FDR < 0.000).

The mean time series of the right hippocampus within the Control group showed significant correlations with proximal regions including the right amygdala, anterior and posterior parahippocampal gyrus, lingual gyrus, and posterior temporal fusiform cortex (peak voxel 30, -16, -18; p-FDR < 0.000). Significant contralateral correlations were obtained in areas of the left hippocampus, and anterior and posterior parahippocampal gyrus (peak voxel -28, -24, -18; p-FDR < 0.000).

Group Comparisons

Consistent with hypothesis 1C, in regions associated with learning and memory, C2 demonstrated decreased functional connectivity when compared to Controls between the right hippocampus and the left cerebellum extending slightly into the left cerebellar vermis (peak voxel -02, -50, -04; p-FDR = 0.031; **Fig. 5**). However, there were no

significant group differences between C1 and C2 or between C1 and Controls for the right hippocampus seed region. Additionally, there were no significant between group effects for functional connectivity of the left hippocampus seed region.

RESEARCH QUESTION 2

Consistent with hypothesis 2, when C1 and C2 were combined into a larger ASD group and subsequently compared to the Control group, none of the 6 regions of interest (right/left middle frontal gyrus, right/left posterior superior temporal lobe, right/left hippocampus) showed a significant group effect for connectivity with any other areas of the brain. The previously identified clusters with between group differences, as described above, were no longer significant in the ASD versus Control comparisons.

Chapter 4: Discussion

Discussion of Findings

This study demonstrated alterations in brain functional connectivity among previously identified neurocognitive subtypes of ASD. Subgroups were identified using a data driven approach to identify unique profiles of neurocognitive functioning. Both subgroups had above average IQ and below average fine motor skills; however, the first subgroup, Cluster 1, also had above average reasoning and language abilities while the second subgroup, Cluster 2, had below average learning and memory.

Results of this study show that, in a cluster of young adult males with ASD and cognitive strengths in reasoning and language skills, there is increased functional connectivity between frontal brain regions associated with reasoning and other areas of the brain, as well as between temporal brain regions associated with receptive language and other areas of the brain. In contrast, a group of young adult males with ASD and cognitive weaknesses in learning and memory demonstrated decreased functional connectivity between hippocampal brain regions associated with learning and memory and other areas of the brain. Importantly, however, when then two ASD subgroups are combined into one larger but more heterogeneous group, these significant subgroup effects disappeared. Thus, this study highlights the importance of elucidating smaller more homogeneous subgroups of ASD and proposes a method for doing so by utilizing a data driven approach to investigating neurocognitive functioning of ASD. Functional imaging results lend further support to the theory of altered brain connectivity in ASD as

a potential mechanism of impairment, yet emphasize the potential for distinct and unique patterns of alterations within subgroups of the ASD population.

LIMITATIONS

Although the current findings offer an important stepping-stone for future research, there are some limitations that must be considered. First, the small sample size and nature of the statistical methods employed in the exploratory clustering analyses precluded us from making many conclusions regarding the significance of the results or generalizations to the ASD population as a whole. Thus, it will be important for future studies to replicate these analyses with larger sample sizes, including individuals with various levels of functioning across various age ranges and of both sexes. There are likely additional cognitive profiles within the ASD spectrum, however because this study only included high functioning ASD subjects, we were unlikely to capture subtypes existing at the lower end of the spectrum. This is a common limitation for studies that include an imaging component, as lower functioning individuals may have difficulty remaining still during the scans or may find the loud noises from the machine intolerable. Additionally, the level of overall cognitive ability of our sample was particularly high. Therefore, it should be noted that there might be qualitative differences between our sample and other samples. Comparison of clusters utilizing brain-imaging data that were not included in the cluster analysis offers an important form of external validation (Aldenderfer, 1985). However, continued verification of potential cognitive subtypes/clusters will be needed.

CONCLUSIONS

Autism spectrum disorder is a heterogeneous neurodevelopmental disorder. Increasing efforts have been made to identify the underlying etiology of this disorder, yet no single cause has been found. Numerous researchers have suggested that difficulties in elucidating the mechanism of impairment in ASD may be due to the heterogeneous nature of the population. While it is widely understood that ASD symptoms are related to altered brain functioning, there is little consistency in the field regarding the mechanisms and location of that impairment. Neurocognitive abilities are also related to brain functioning and neuropsychologists have been measuring and studying mechanisms related to neurocognitive functioning for many years. Standardized neuropsychological tests provide an instrument for directly assessing brain-behavior relationships and localization of functioning, and thus provide a window into the functioning of the human brain. Identification of neurocognitive profiles has provided great diagnostic utility for other disorders, such as allowing for better differentiation between vascular dementia versus Alzheimer's. Researchers have attempted to similarly identify a common neuropsychological profile of ASD, yet have failed to identify a singular profile that holds true for individuals across the heterogeneous spectrum of impairment.

Neuroscientists now understand that brain function and dysfunction rarely occurs in isolation. Instead, the brain is comprised of billions of connections both locally, within proximal structures, and distally, across distinct structures and on opposite sides of the brain. Functional connectivity imaging is a newly emerging tool for better understanding the functional networks within the brain. Many studies have suggested that ASD may be

associated with increased local connectivity in conjunction with decreased distal connectivity, yet there is currently no consensus in the field as other researchers have also reported opposite findings. Again, the difficulty in achieving consensus within the field of ASD research has long been associated with the significant heterogeneity of the population. Perhaps, both sides of the proverbial connectivity-story are true, yet only within unique and differing subgroups within the ASD population. For example, if there is a subset of children with ASD for whom the underlying etiology is "Gene A" with an associated mechanism of impairment in "Brain Region B," these finding will be blurred when mixed in with a group of children who have different etiologies or different mechanisms of impairment.

This study provides a data driven method for parsing a heterogeneous group of individuals with ASD into smaller and thus more meaningful subgroups using neurocognitive performance from commonly used standardized neuropsychological test instruments. The study then continues further by examining underlying brain functional connectivity among identified subgroups in order to validate the meaningfulness of neurocognitive subtypes and beginning to explore differential underlying mechanisms of brain functioning. Finally, this study highlights the negative impact of using a combined ASD group, which may blur meaningful effects and skew results. As such, future research should continue to explore methods for elucidating homogenous subtypes of ASD. In conjunction with increased effort to identify subgroups of ASD, researchers should be conscientious of the impact of sample heterogeneity on their results and make efforts to ameliorate such impact.

Appendices

APPENDIX A: NEUROCOGNITIVE SUBTYPES OF ASD: A REVIEW OF THE LITERATURE

Description and Diagnosis of ASD

Autism Spectrum Disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder that is estimated to occur in approximately 1 in every 68 children (CDC, 2014), representing a significant increase from previous estimates. ASD effects individuals from all racial, ethnic, and socioeconomic backgrounds; however, there is a disproportionate rate of diagnosis in boys versus girls, with prevalence estimates of 1 in every 42 boys in contrast to 1 in every 189 girls receiving an ASD diagnosis (CDC, 2014). Given the high prevalence of ASD, it is among one of the most economically impactful developmental disabilities in our society with estimated total medical costs of $11.5 billion to $60.9 billion in the United States alone (Buescher, Cidav, Knapp, & Mandell, 2014). These estimates include a variety of costs including medical care, special education, and impact on parents' productivity. Treatment including intensive behavioral interventions, such as applied behavior analysis, costs an average of 40 thousand to 60 thousand dollars per child, annually (Amendah, Grosse, & Bertrand, 2011). These interventions are well supported in the research literature, yet there currently is no known cure for ASD (Weitlauf et al., 2014).

The constellation of deficits was originally described by Kanner in 1943 and was initially termed infantile autism (Kanner, 1943). One year later, Hans Asperger published a description of autistic children who had lacked the cognitive impairment and language

dysfunction described by Kanner (Asperger, 1943). However, the term Asperger's disorder was not used until many years later, when Dr. Lorna Wing coined the term in a paper of her own in 1981 (Wing, 1981). Diagnostic descriptions of the disorder have undergone significant revision since the original differentiation from childhood schizophrenia that was described the DSM-III. The most recent diagnostic revisions came with the publication of the current edition of the Diagnostic and Statistical Manual of Mental Disorders-5th Edition (DSM-5). Currently, diagnostic criteria of ASD include a pattern of persistent impairment in social communication and social interaction in concert with restricted, repetitive patterns of behavior or interests (American Psychiatric, 2013). In addition to changes in the behavioral symptoms necessary to meet criteria for the disorder, the most significant change is arguably the change in the name of the disorder itself. Previously, under the DSM-IV TR, ASD was used as an umbrella term that encompassed multiple "subtypes" of pervasive developmental disorders. These subtypes included autistic disorder, Asperger's disorder, and pervasive developmental disorder - not otherwise specified (PDD-NOS) (American Psychiatric, 2000). Each subtype of pervasive developmental disorder had a similar, but distinct set of diagnostic criteria. Notably, both Asperger's disorder and PDD-NOS lacked the criteria of language impairment. Now, using the DSM-5, there is only one defined disorder, Autism Spectrum Disorder (ASD), to represent the range of impairment previously classified under pervasive developmental disorders (American Psychiatric, 2013). Using the DSM-5, some clarification can be provided by the use of diagnostic specifiers (i.e., requires support, requires substantial support, or requires very substantial support). Additionally,

clinicians can specify either with or without intellectual disability. However, interpretation of these specifiers, and of what constitutes classification into one versus another, is somewhat ambiguous. There has been some concern and critique from the community, researchers, and professionals regarding these diagnostic changes. Specifically, there is concern regarding the potential for individuals who were previously diagnosed with Asperger's disorder or PDD-NOS under the DSM-IV TR to lose their diagnosis due to a lack of required language impairment despite otherwise meeting diagnostic criteria (Parsloe & Babrow, 2015). In justification of the revisions, the authors of the DSM-5 cite the lack of research supporting a clear distinction between the DSM-IV subtypes. For example, previous studies have described the ambiguity between Asperger's disorder and high-functioning autistic disorder, with the same symptom presentation receiving a different diagnosis from one clinician to the next (C. Lord et al., 2012). Additionally, numerous brain imaging studies have failed to demonstrate significant differences between Asperger's disorder and high-functioning autistic disorder (Pina-Camacho et al., 2012). In fact, it has become common methodological practice for researchers to combine the Asperger's disorder and high functioning autism subtypes into one group with which to compare to a typically developing control group. Given the difficulty of differentiating between subtypes in both research and clinical practice, the authors of the DSM-5 took a step back, so-to-speak, and thus paved the way for researchers to develop new ways to classify ASD into more stable and meaningful subtypes (R. Grzadzinski, M. Huerta, & C. Lord, 2013).

Etiology of ASD

Despite decades of research, there is no known cause of ASD (Abrahams & Geschwind, 2010; Geschwind, 2011). There have been significant advances towards this goal in recent years as researchers have begun to identify candidate genes and genetic variations that contribute to ASD symptomology (Egger et al., 2014). There is some agreement within the field on the contribution of both genetic as well as environmental factors that likely interact with each other to contribute to ASD pathology (Gardener et al., 2009; Hunter, 2005). Evidence for genetic heritability has been well established with twin studies (Ronald et al., 2006). Recent reports have demonstrated heritability rates of over 80% (Ronald & Hoekstra, 2011). In addition, advances in technology have lead to the identification of over 250 associated genes; though, no single gene accounts for more than 1-2 percent of ASD cases (Ellegood et al., 2015). Although the exact cause of ASD is unknown, it is well understood that ASD is a neurocognitive disorder with evidence for disrupted cortical structure and function.

Subtyping ASD

Researchers have argued the benefit of parsing the heterogeneity of ASD into more homogenous subtypes (Rebecca Grzadzinski et al., 2013; Happé et al., 2006; Lai et al., 2013). A common methodological approach has been to use cluster analysis in order to determine group membership without specifying diagnoses *a priori*, thus classifying subjects empirically based on quantitative data. Researchers have utilized cluster analysis and other multivariate approaches in attempting to determine the number and nature of ASD subtypes based on behavioral symptoms (Constantino et al., 2004; Hu & Steinberg,

2009) (Ring, Woodbury-Smith, Watson, Wheelwright, & Baron-Cohen, 2008), cognitive measures (D. Fein, L. Waterhouse, D. Lucci, & D. Snyder, 1985a; Lewis et al., 2007; Rapin et al., 2009), brain imaging data (Michal Hrdlicka et al., 2005), or a mix of these data types (Ben-Sasson et al., 2008; Bitsika, Sharpley, & Orapeleng, 2008; Lane, Dennis, & Geraghty, 2011). Results have been inconsistent, with data supporting models with two (Stevens et al., 2000), three (Bitsika et al., 2008; Wiggins, Robins, Adamson, Bakeman, & Henrich, 2012), four (M. Hrdlicka et al., 2005; Sacco et al., 2012), or more subtypes (Fein et al., 1985a; Lane, Young, Baker, & Angley, 2010; Lecavalier, 2006). Additionally, the nature of ASD subtypes has also been inconsistent; with some researchers finding support for distinct phenotypic subtypes (Bruining et al., 2010; Hu & Steinberg, 2009; Rapin et al., 2009) and others arguing that ASD clusters represent a severity gradient (Constantino et al., 2004; Stevens et al., 2000; Wiggins et al., 2012).

Numerous studies have been dedicated to better understanding the range of behavioral, social, and communication functioning across the spectrum of ASD, yet there is a paucity of research examining variations of cognitive abilities within this population. Although the importance of assessing cognitive ability in individuals with ASD has been well documented (Frith, 2012; Happé & Frith, 1996), only a few of the studies listed above have included measures of cognitive functioning in the clustering algorithm. When these data were included, they were often limited to a few cognitive domains [e.g., general intellectual ability (IQ), measures of language functioning (Fein et al., 1985a; Lewis et al., 2007; Rapin et al., 2009). Results from studies using single broad measures of cognitive functioning (e.g., FSIQ) or only measures from one domain of functioning

(e.g., language) may be somewhat misleading due to the limited scope of cognitive data included in the models.

The most comprehensive attempt to cluster ASD subjects based on unique cognitive profiles was conducted by Fein and colleagues in 1985. This study utilized a hierarchical cluster analysis to group 54 children with ASD, ages 5-17 years old, using four composite scores from the McCarthy Scales of Children's Abilities (Verbal, Perceptual Performance, Quantitative, and Memory) and the Peabody Picture Vocabulary Test (PPVT) as an additional measure of language ability. Results from this study suggested that an eight-cluster solution provided the best fit. Approximately half of the children were clustered into three groups, with peaks on perceptual-performance tests. Two clusters had peaks on verbal tests. Two clusters had more complex patterns of inter-test scatter and one cluster had minimal scatter demonstrating a profile of impairment across domains included in the analyses (D. Fein, L. Waterhouse, D. Lucci, & D. Snyder, 1985b).

The Cognitive Profile of ASD

The heterogeneity of cognitive abilities within ASD has been well documented. Variability in etiology and the course of brain development may play a role in the variability of cognitive functioning within this population. Individuals with ASD have a diverse range of cognitive abilities and disabilities and thus, attempting to define one single cognitive profile of ASD may not be realistic. In fact, no single model that has been put forth has fully captured the range of heterogeneity in the population.

The "Theory of Mind" cognitive model is an attempt to explain impairments in social communication (Baron-Cohen, Leslie, & Frith, 1985), yet this model does not account for stereotypical movements and repetitive interests. The "Executive Dysfunction" cognitive model is used to explain repetitive interests and lack of generativity (Ozonoff, Pennington, & Rogers, 1991), yet not all individuals with ASD demonstrate cognitive impairments in executive functioning. Some researchers have described ASD as being defined by a "weak central coherence" (Happé & Frith, 2006), though this explanatory model is limited to non-social deficits in ASD. Attempts at finding a unitary cognitive model to explain the heterogeneous range of impairment in ASD have been largely unsuccessful (Charman et al., 2010). This difficulty may be partially attributed to the fact that these unitary models of cognitive profiles have often been described by mean deficits across a heterogeneous sample, thus obscuring different patterns of spared/impaired cognitive functioning that may exist in subgroups of individuals with ASD.

Brain Imaging

Localized Function

The advent of increasingly sophisticated neuroimaging techniques has led to significant advances in the understanding of brain function. Magnetic resonance imaging (MRI) is a non-invasive neuroimaging technique that allows for in-vivo examination of structural brain components, connections throughout the brain, and brain functioning. Additionally, it is suitable for use in children and clinical populations (Raschle et al., 2009). As the literature base within the neuroimaging field continues to rapidly grow,

researchers are increasingly better able to explore the relationships between the brain and behavior.

The concept of localized brain function began as early as the 1800's and further support for localization of brain function has been provided through lesion studies, including a seminal description by Paul Broca of a patient with a lesion in the left inferior frontal cortex who presented with significant expressive language impairment (Broca, 1861). There have since been thousands of cases of brain lesions and corresponding neurocognitive impairment resulting in well-established patterns of brain function related to specific brain structures (Cabeza & Nyberg, 2000). Another widely cited study is that of patient H.M. who lost his ability to form new memories following surgical ablation to bilateral hippocampal regions (Scoville & Milner, 1957). Additionally, localization of executive functions to frontal brain regions has been frequently associated with Phineas Gage, a patient who suffered significant damage to frontal regions following an accidental impaling from an iron rod (Harlow, 1868).

The relationship between brain regions and corresponding neurocognitive function has been further validated through the use of functional MRI (fMRI), which detects changes in hemodynamics necessary to support brain neural activity. Specifically, changes in a subject's blood oxygen level dependent (BOLD) signal in specific brain regions seen while the subject is performing some task can provide evidence for localized neural activation (Kim, 2007). For example, fMRI studies of typically developing subjects consistently report activation of the contralateral pre-central gyrus during simple motor tasks, such as tapping a finger or pushing a button while in the scanner. For review

of functional localization, see Cabeza & Nyberg (2000). However, the over-simplification of functional localization is becoming increasingly apparent within the field. In Cabeza and Nyberg's review of 275 imaging studies, despite converging evidence for localization of function, they provide support for the importance of brain networks due to the fact that all task performance seemed to rely on multiple brain regions (Cabeza & Nyberg, 2000).

Brain Networks

Network approaches to the study of brain functioning have become increasingly popular (Matthews & Fair, 2015). Advances in imaging techniques, such as the ability to examine network structure and function, have aided this shift. Diffusion tensor imaging (DTI) is one of the most common methods for studying the structure of brain networks. Specifically, DTI allows for examination of the architecture and integrity of white matter tracts by measuring the magnitude and orientation of the diffusion of water molecules in biological tissue (Basser, Mattiello, & LeBihan, 1994). The most common measurements derived from DTI data include mean diffusivity and fractional anisotropy. Typically, abnormalities of structural connectivity are represented as increased mean diffusivity and reduced fractional anisotropy (Basser et al., 1994). DTI is useful for in vivo imaging of the structural components of connections within and between brain networks (Khanna, Altmeyer, Zhuo, & Steven, 2015). However, it is important to note that these measurements are only an estimation of true axon structure, which currently can only be verified via examination of post-mortem tissue samples (Khanna et al., 2015).

In contrast, functional connectivity MRI (fcMRI) involves the evaluation of brain circuits through identification of common patterns of neural activation across the brain. Specifically, fcMRI research has revealed a coherence in low-frequency fMRI signal between spatially distinct regions in the brain. These patterns of functional coherence have been shown to correspond to known structural networks [For review, see: (Lee, Smyser, & Shimony, 2013)]. In 1995, Biswal and colleagues were the first to examine functional connectivity of brain regions using fMRI. Their findings revealed a functional coherence of the left and right motor cortex to the supplementary motor area while subjects were in a resting state, thus demonstrating the utility of this imaging technique (Biswal, Yetkin, Haughton, & Hyde, 1995). Functional connectivity findings have demonstrated considerable reliability and reproducibility across studies, scans, and days (Damoiseaux et al., 2006) despite many methodological variations in data collection and processing (Biswal et al., 2010). Thus, measurements of functional connectivity serve as a proxy for assessing the extent to which various brain regions work in concert with each other and the extent to which brain functioning occurs within network circuits across the brain. This coherence in BOLD signal has been established in both task-based fMRI studies as well as when subjects are simply at rest. Resting state functional connectivity MRI (rs-fMRI) has become an increasingly popular tool for in-vivo examination of brain networks in clinical populations (Allen et al., 2007; Greicius, Srivastava, Reiss, & Menon, 2004; Tononi & Edelman, 2000) as well as in children (Fair et al., 2008; Kelly et al., 2009). This is partially due to the lack of necessity for task performance in the

scanner, which can be methodologically challenging in clinical populations such as children with ASD (Matthews & Fair, 2015; Muller, 2007).

Connectivity in ASD

This shift towards examination of brain networks rather than localized areas of abnormality has impacted how researchers conceptualize and examine abnormal brain functioning. Specifically, within the field of autism research, there has been increasing support for the theory of disrupted neural networks rather than specific localized brain abnormalities (Muller, 2007; Rane et al., 2015). Unfortunately data from structural and functional imaging studies of ASD are often inconsistent, despite the trend toward network level examination of brain abnormalities. While some of these inconsistencies may be related to methodological differences in both data collection and data processing, there are likely also population variables that should be taken into consideration (Muller et al., 2011; Nair et al., 2014). Given the well-established heterogeneity of ASD, controlling for these population related variables has presented significant challenges in the field. The current literature base regarding brain network connectivity in ASD will be reviewed below.

Structural Connectivity

Diffusion tensor imaging has been carried out on a wide range of ages within the ASD population, from as young as 2-years-old (Walker et al., 2012) to adults in their 50th decade of life (Bloemen et al., 2010). Studies also varied with respect to gender ratio and DSM diagnosis of participants. As discussed earlier, it is common for studies to combine participants with high-functioning autism, Asperger's, and PDD-NOS into one ASD

group, purportedly representing ASD as a whole. However, the proportion of respective DSM-IV subtypes varies from study to study. In general, increased mean diffusivity in ASD versus typically developing control subjects and reduced fractional anisotropy in ASD versus typically developing control subjects were the most commonly reported findings in DTI studies. Specifically, this pattern of increased mean diffusivity and reduced fractional anisotropy in ASD relative to control subjects was reported broadly in bilateral frontal, temporal, and parietal lobes (Groen, Buitelaar, van der Gaag, & Zwiers, 2011; Shukla, Keehn, & Muller, 2011), as well in specific white matter tracts including corpus callosum, superior longitudinal fasiculus, inferior fronto-occipital uncinate fasiculus, cingulum, and internal capsule [For review: (Rane et al., 2015)]. Of particular relevance, these white matter tracts are widely implicated in cognitive functions including language, memory, and executive functions.

The pattern of findings in these DTI studies may suggest reduced integrity of white matter tracts in the ASD group relative to controls. However, the opposite pattern was also reported with findings of decreased mean diffusivity (Bloemen et al., 2010; Sahyoun, Belliveau, Soulieres, Schwartz, & Mody, 2010) and increased fractional anisotropy (Beacher et al., 2012; Billeci, Calderoni, Tosetti, Catani, & Muratori, 2012; Brito et al., 2009; Sivaswamy et al., 2010) in ASD relative to controls. Additionally, there have been a number of reports of no group differences in mean diffusivity (Brito et al., 2009) and fractional anisotropy (Ameis et al., 2011; Joseph et al., 2014; Nagae et al., 2012; Nair, Treiber, Shukla, Shih, & Muller, 2013). In order to move towards a clearer understanding of brain abnormalities in ASD and the underlying mechanisms, it will be

important for researchers to consolidate findings and reconcile inconsistencies in the literature.

Functional Connectivity

One prominent theory regarding ASD is that symptoms are related to abnormalities in the connections between cortical brain regions (Just, Cherkassky, Keller, & Minshew, 2004; Kana, Libero, & Moore, 2011). Specifically, Just and colleagues reported under-connectivity between brain regions related to complex cognitive processing in ASD relative to typically-developing control subjects (2004). Other researchers have since revised the cortical under-connectivity theory of ASD and described the disorder as being defined by "disrupted cortical connectivity" in order to encompass emerging evidence for both over connected as well as under connected brain regions (Belmonte et al., 2004; Kana et al., 2011). Specifically, research has begun demonstrating patterns of under connectivity between long-range cortical to cortical regions as well as over connectivity between short-range cortical connections (Mueller et al., 2013). It has been theorized that localized over connectivity might serve as a compensatory mechanism for under connectivity between long-range cortical regions (Muller et al., 2011). However findings of regional over connectivity in conjunction with long-range under connectivity have not been consistently reported (Picci et al, 2016).

With respect to studies of functional connectivity in ASD, there is perhaps even less consensus in the literature than there is for studies of structural connectivity. However, the most consistent finding is that of reduced cortico-cortical functional connectivity in ASD groups relative to control groups in prefrontal cortical regions [For

review: (Rane et al., 2015)]. The prefrontal cortex is an area of early overgrowth followed by abnormal pruning throughout brain development in children with ASD (Courchesne, Campbell, & Solso, 2011). Thus, the impacts of altered brain development in this region may result in the observed abnormal connectivity. Additionally, disrupted long-range functional connectivity has been reported in pre-central cortex, anterior cingulate cortex, superior temporal gyrus, insula, and precuneus (Rane et al., 2015).

In summary, significant research has focused on identifying ways in which the brains of individuals with ASD may deviate from normal development. Clarification of cognitive dysfunction within the population of ASD is important for treatment efforts. However, no single cognitive profile of ASD captures the range of heterogeneity with the population. Heterogeneity of ASD has been well documented and researchers have called for further elucidation of smaller, and more meaningful subtypes in order to help clarify the etiology of ASD and underlying brain mechanism. Elucidation of meaningful cognitive subtypes would help advance the field of research and may provide greater understanding of underlying brain abnormalities. Thus, it is important to continue to explore potential subtypes of ASD and related differences in underlying brain mechanisms. Functional connectivity MRI is a non-invasive tool that can help identify patterns of functional network abnormalities between different areas in the brains of individuals with ASD. The ASD neuroimaging field is working towards consolidation of findings and elucidation of mechanisms related to patterns of abnormal network connectivity. However, inconsistencies in findings are still very common. As such, exploration of differing functional connectivity patterns between potential neurocognitive

subtypes is warranted. Future research should continue to explore additional methods for parsing ASD into more homogenous diagnostic groups.

APPENDIX B: NEUROCOGNITIVE SUBTYPES OF ASD: A PRELIMINARY STUDY

Preliminary Clustering Analyses

Data from all neuropsychological measures were transformed into z-scores based on published test norms when available or on norms derived from the control sample when test norms were not available. These z-scores were then combined into composite scores reflecting each domain of interest, resulting in 12 composite mean z-scores. Within each domain, correlations between z-scores ranged from moderate to high (r = .408 to .912). To determine whether unique profiles of neuropsychological functioning exist among individuals with ASD, as well as the nature of these potential subgroups, *k*–means cluster analysis was then performed.

While the *k*–means procedure is commonly used in this type of analysis, as it is less susceptible to outliers and the inclusion of potentially non-relevant variables, one limitation is the requirement to indicate the number of clusters (*k*) to be extracted *a priori* (Aldenderfer, 1985). Because this study was exploratory in nature and the intent was to identify the presence and nature of *potential* subtypes, the appropriate number of clusters was unknown. Therefore, an initial agglomerative hierarchical clustering algorithm was run using Ward's minimum variance method and squared Euclidean distance as the measure of difference between clusters. In order to determine the number of clusters to be extracted during the *k*–means clustering procedure, the dendrogram resulting from the hierarchical analysis was examined for gaps in distance measurements between clusters using procedures outlined in the SPSS manual (SPSS, 2010). Finally, the nature of the clusters identified during the *k*–means cluster analysis was defined by examining the

means of the final cluster centers for each of the 12 domains. The relative importance of each composite domain score in the final cluster solution was determined using analysis of variance (ANOVA) techniques. SPSS statistical software package version 19.0 was used for all statistical analyses (SPSS, 2010).

Preliminary Clustering Results

Based on examination of the dendrogram resulting from the initial hierarchical cluster analysis, the three-cluster solution was selected as providing the best separation between clusters. Next, a *k*–means cluster analysis was run with initial cluster centers randomly generated and up to 20 iterations allowed. Final cluster centers were achieved with convergence after three iterations. See **Table C3** for distances between final cluster centers. Mean domain scores for each cluster are illustrated in **Figure C1**. Of the total sample of 20 participants, nine belonged to cluster 1 (C1); nine belonged to cluster 2 (C2); and two belonged to cluster 3 (C3).

Descriptions of clusters are based on their profile of apparent strengths and weakness, defined as mean domain scores greater than .67 standard deviations above or below the mean. This cutoff score was selected to correspond to standard scores above 110 or below 90, which is a commonly used criterion in clinical assessments to differentiate between average and above average or below average performance (Guilmette, Hagan, & Giuliano, 2007). Analysis of variance (ANOVA) on domain scores for each cluster provides additional information about the relative importance of each domain score to the final cluster solution by comparing the *F* statistics. Importantly, however, significance values are useful for descriptive purposes only and cannot be used

to extrapolate to population differences, as they were derived from a clustering algorithm designed to optimize differences between clusters.

C1 included subjects with strengths in general intellectual ability (FSIQ) and reasoning, high average receptive and expressive language, and a weakness in fine-motor skills. C2 was defined by subjects with high FSIQ scores and low scores on measures of verbal learning and memory, visual learning and memory, and fine-motor skills. Generally, performance across domains appeared more varied in this cluster. C3 was comprised of 2 subjects with relatively low performance on tests of reasoning ability, cognitive flexibility, working memory, verbal learning and memory, expressive and receptive language, and visuospatial processing. While these 2 subjects may appear to be outliers, the small sample size precluded us from making determinations about their representativeness to the population and therefore they were not removed from these analyses. Percentages of subjects in each cluster meeting criteria for autism versus autism spectrum based on ADOS criteria is described in **Table C4**.

Examination of the resulting ANOVA table (see **Table C1**) suggests the reasoning domain scores provided the greatest separation between clusters and thus contributed most heavily to the final cluster solution, followed by performance on tests of receptive language and verbal learning and memory. Interestingly, performance on measures of social and emotional processing was less useful for determining the final cluster solution and subjects tended to perform in the average range across the clusters in this domain.

APPENDIX C: TABLES AND FIGURES

Domain	Cluster		Error		F	Sig.
	Mean Square	df	Mean Square	df		
Attention	.574	2	.541	15	1.061	.371
Reasoning	8.089	2	.220	17	36.792	.000
Cognitive Flexibility	2.492	2	.606	16	4.113	.036
Working Memory	4.642	2	.395	17	11.758	.001
Visual Learning/Memory	2.682	2	1.081	15	2.481	.117
Verbal Learning/Memory	9.761	2	.636	17	15.340	.000
Expressive Language	6.057	2	.646	17	9.373	.002
Receptive Language	5.440	2	.292	17	18.613	.000
Visuospatial	2.119	2	.201	15	10.561	.001
Fine Motor	2.127	2	1.549	17	1.373	.280
Social/Emotional	2.091	2	1.185	14	1.765	.207
Full Scale IQ (FSIQ)	2.900	2	.354	17	8.192	.003

Table C1. ANOVA results indicating extent to which domains contributed to the final clustering solution. Higher F values correspond to greater contribution.

Domain / Composite	Test	Score
Attention	Conner's Continuous Performance Test, Second Edition (CPT II V.5)	Percent commissions raw score
		Percent omissions raw score
Reasoning	Woodcock Johnson Tests of Cognitive Abilities, Third Edition (WJ Cog III)	Analysis-Synthesis raw score
		Concept Formation raw score
	Wechsler Abbreviated Scales of Intelligence (WASI)	Matrix Reasoning total raw score
Cognitive Flexibility	Wisconsin Card Sort Test (WCST)	Perseverative Errors
		Nonperseverative Errors
Working Memory	Wechsler Adult Intelligence Scale (WAIS-IV)	Digit Span Backwards raw score
		Digit Span Sequencing raw score
Visual Learning and Memory	Brief Visuospatial Memory Test-Revised (BVMT-R)	Total recall raw score
		Delayed recall raw score
Verbal Learning and Memory	Hopkins Verbal Learning Test-Revised (HVLT-R)	Total recall raw score
		Delayed recall raw score
Expressive Language	Wechsler Abbreviated Scales of Intelligence (WASI)	Vocabulary total raw score
	Boston Naming Test, Second Edition (BNT)	Total raw score
Receptive Language	Token Test	Total raw score
	Peabody Picture Vocabulary Test, Fourth Edition (PPVT)	Total raw score
Visuospatial	Judgment of Line Orientation (JLO)	Total number correct
Fine Motor Coordination	Grooved Peg Board	Dominant hand completion time
		Non-dominant hand completion time
Social and Emotion Processing	Wechsler Advanced Clinical Solutions-Social Cognition (ACS-Social Cog) Social Perception	Total raw score
General Cognitive Ability	Wechsler Abbreviated Scales of Intelligence (WASI)	Full-scale IQ (FSIQ)

Table C2. Neuropsychological tests and scores included in the clustering analyses.

Cluster	1	2	3
1		3.052	6.648
2	3.052		4.970
3	6.648	4.970	

Table C3. Distances between final cluster centers for the *k*-means cluster analyses.

			Cluster Number		
			1	2	3
ADOS Classification	Autism	N	2	4	2
		% of Cluster	22.2%	57.1%	100.0%
	Autism Spectrum	N	7	3	0
		% of Cluster	77.8%	42.9%	0%
Total		N	9	7*	2

*ADOS Classification data are missing for two subjects from Cluster 2.

Table C4. Percentages of ADOS classifications by cluster.

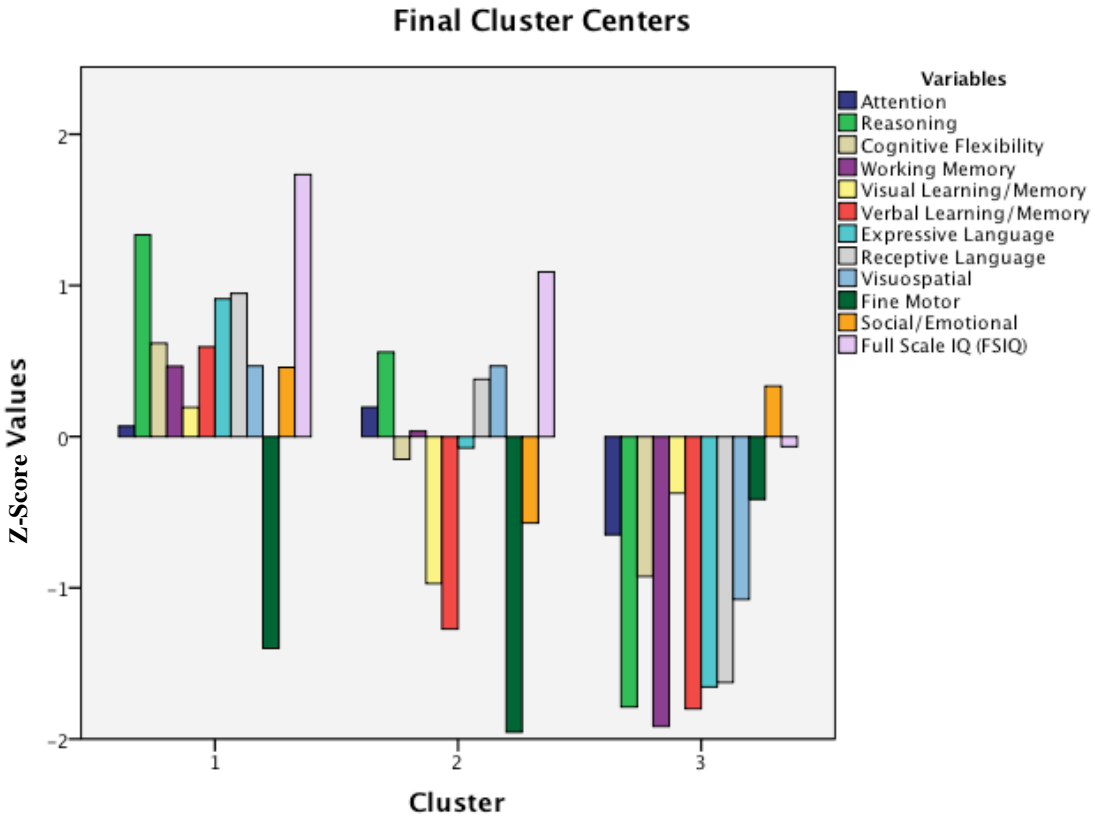

Figure C1. Mean domain z-scores for each cluster.

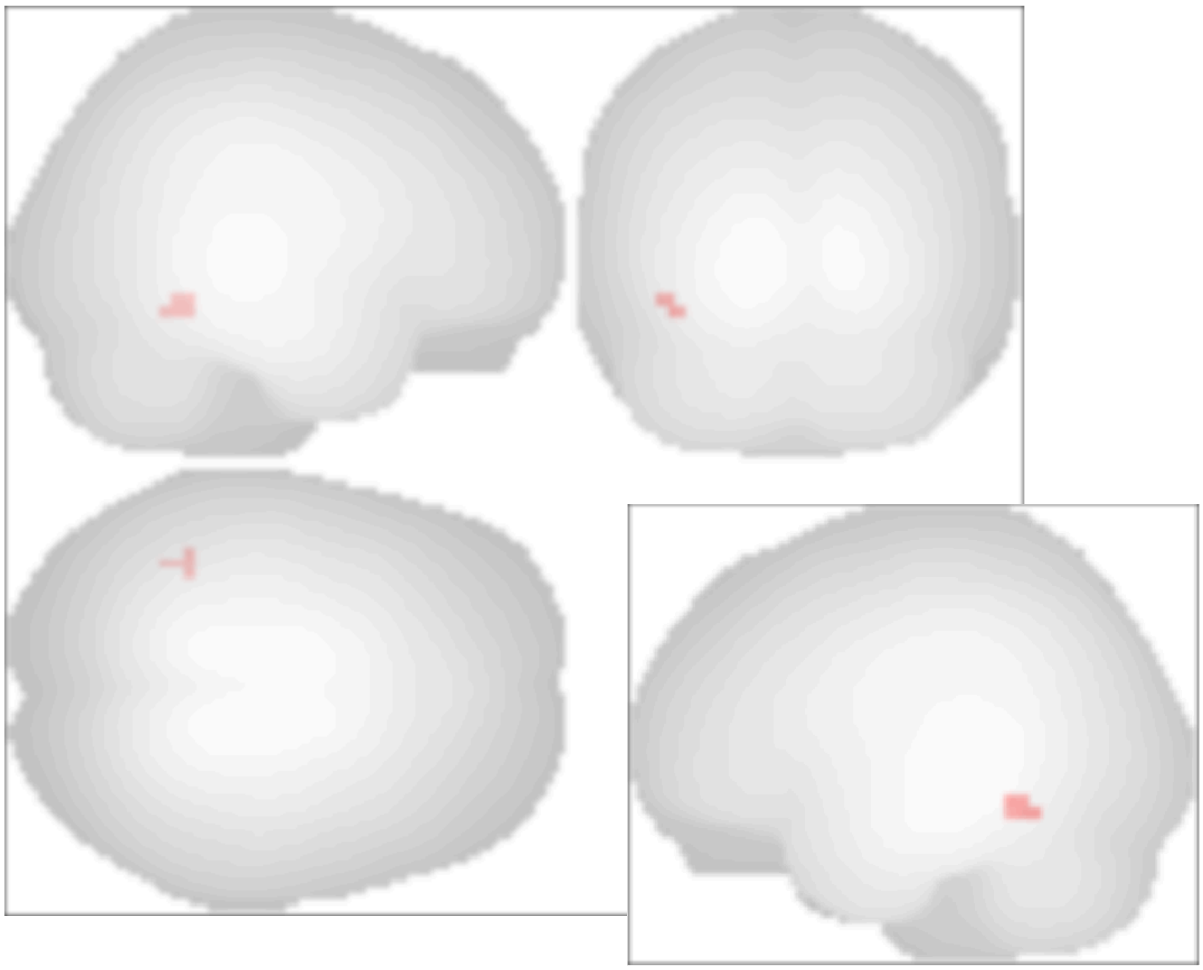

Figure C2. Area of C1 > Controls functional connectivity for the left middle frontal gyrus seed region.

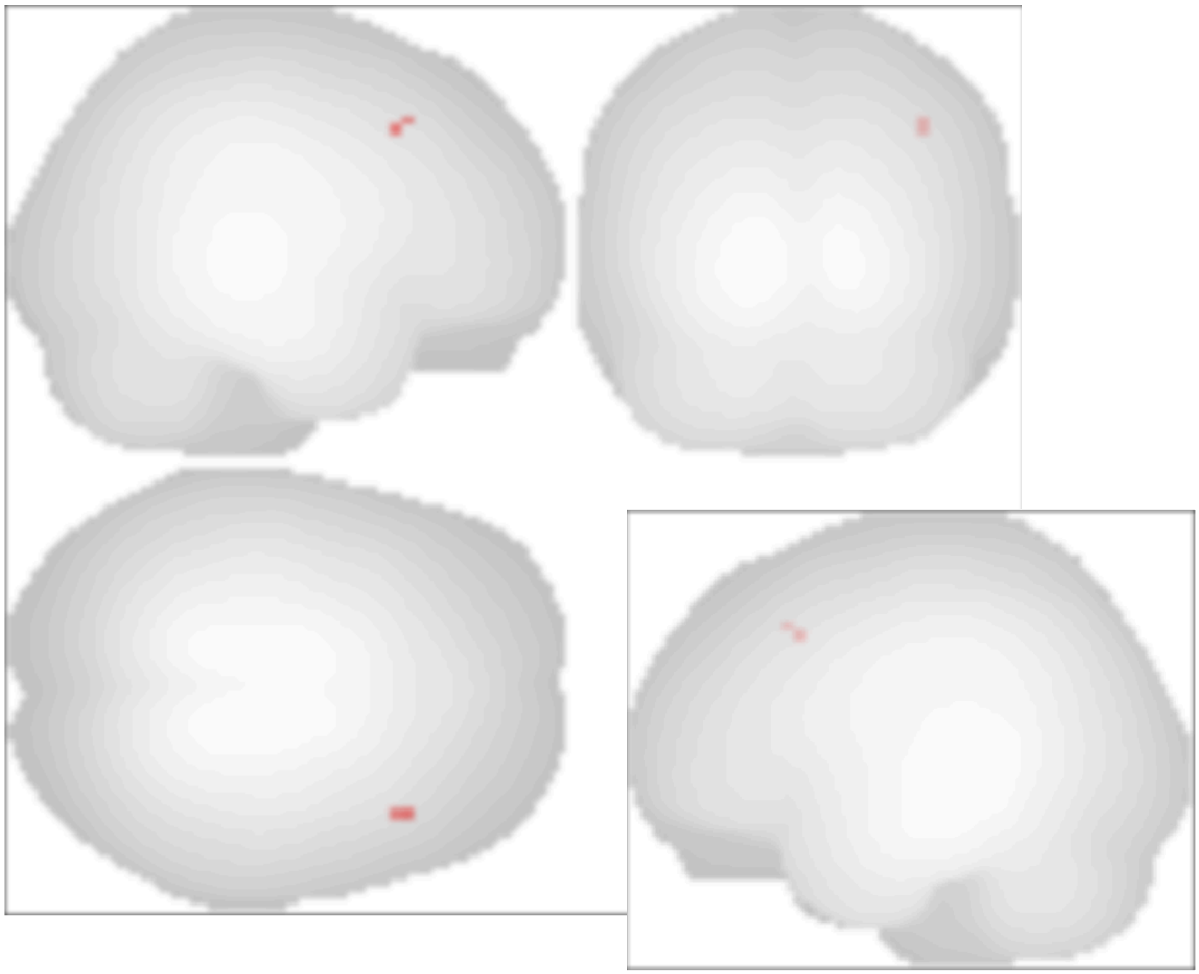

Figure C3. Area of C1 > C2 functional connectivity for the left middle frontal gyrus seed region.

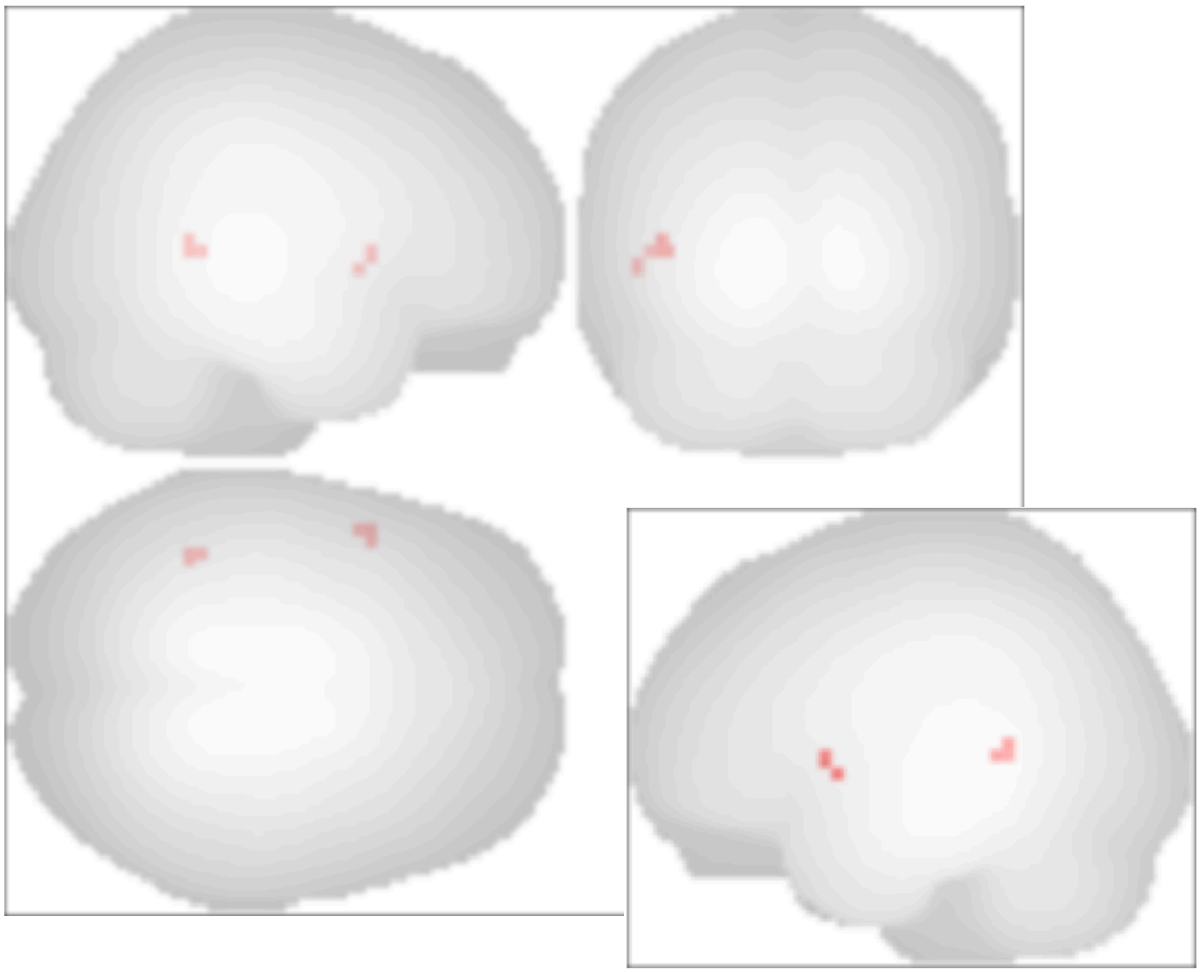

Figure C4. Areas of C1 > C2 functional connectivity for the right posterior superior temporal gyrus seed region.

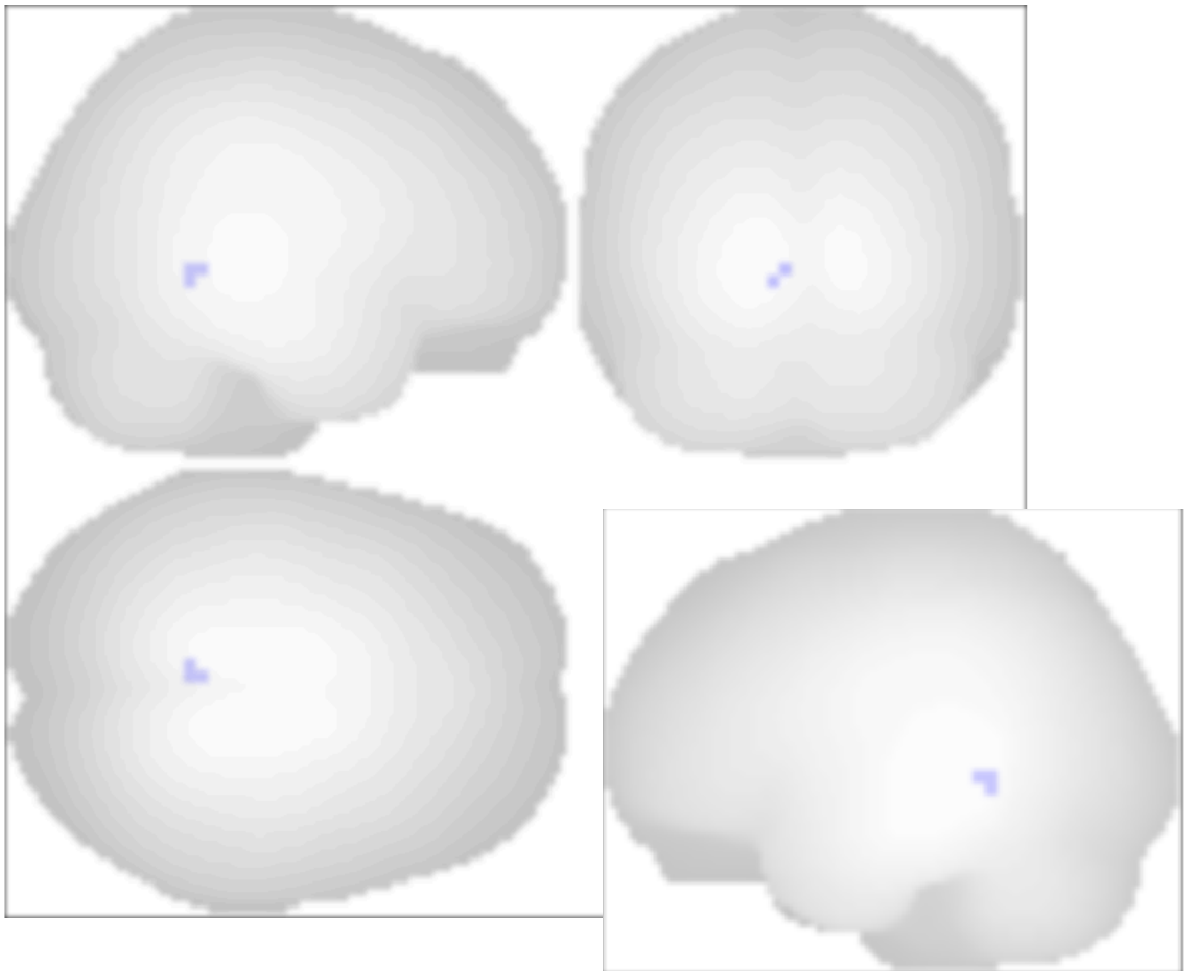

Figure C5. Area of C2 < Controls functional connectivity for the right hippocampus seed region.

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